

Figure S1. Size-exclusion chromatography of Orc1 variants. All Orc1 mutants expressed at similar levels as the WT initiators, and eluted from a final size-exclusion column as sharp, symmetrical, monomer peaks, with retention times identical to WT Orc1-1 and Orc1-3. Chromatography traces from a subset of Orc1-1 and Orc1-3 mutants are shown for comparison with the WT initiators.

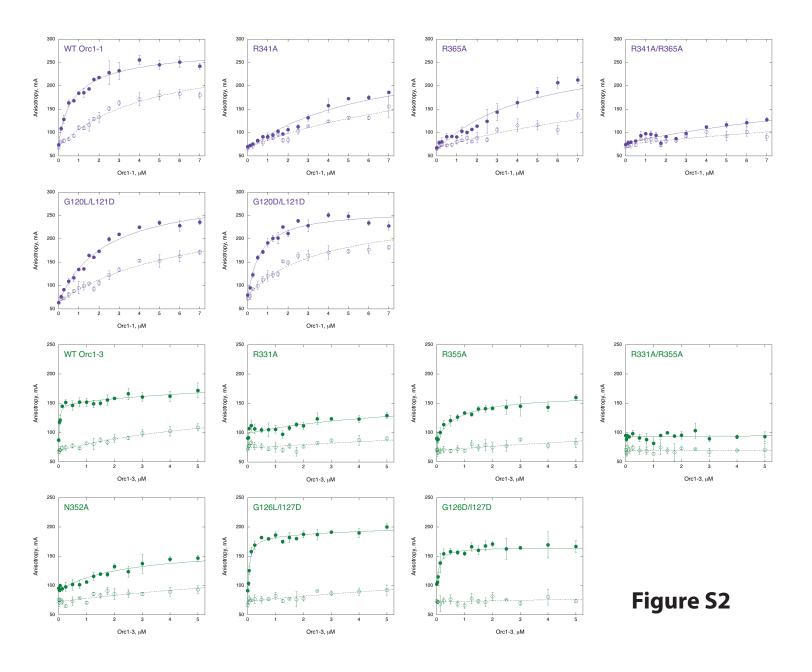


Figure S2. Orc1 binding curves. Binding to a specific origin site (closed circles) versus a randomized DNA sequence (open circles) is shown for each Orc1-1 (purple) and Orc1-3 (green) variant. The specific origins sites used for Orc1-1 and Orc1-3 are the mORB and C3 site sequences, respectively. Data shown are averaged from triplicate experiments and fit as described in the main text.